

Application No. 10/540,086  
Paper Dated: May 5, 2008  
In Reply to USPTO Correspondence of March 5, 2008  
Attorney Docket No. 4544-051936

**AMENDMENTS TO THE CLAIMS**

This listing of claims will replace all prior versions and listings of claims in the application.

**Listing of Claims**

Claims 1-24 (canceled).

Claim 25 (previously presented): Oligonucleotide primers for specific amplification of a *hupB* gene of *Mycobacterium* species selected from the group consisting of Seq ID No. 1, Seq ID No. 2, Seq ID No. 3, Seq ID No. 4 and Seq ID No. 5.

Claim 26 (previously presented): A method for differentiating *Mycobacterium* species based on target gene encoding for histone like proteins such as *hupB* comprising:

- a) obtaining DNA from culture or from clinical samples;
- b) amplifying a part of the target gene encoding for histone like proteins such as *hupB* of *Mycobacterium* species using said DNA as a template in a polymerase chain reaction with a pair of oligonucleotide primer according to claim 25; and
- c) detecting said amplified fragment of the *hupB* gene to detect the presence of *Mycobacterial* species or not and differentiating *Mycobacterium tuberculosis* from *Mycobacterium bovis* based on the size of the amplified fragment.

Claim 27 (previously presented): The method according to claim 26, wherein said *Mycobacterium tuberculosis*, and or *Mycobacterium bovis* species is selected from a group of genetically related *Mycobacteria* and from unrelated microorganisms.

Claim 28 (previously presented): The method according to claim 26, wherein the pair of oligonucleotide primers comprises Seq ID No. 1 and Seq ID No. 2.

Claim 29 (previously presented): The method according to claim 26, wherein the pair of oligonucleotide primers comprises Seq ID No. 3 and Seq ID No. 2.

Claim 30 (previously presented): The method according to claim 26, wherein the pair of oligonucleotide primers comprises Seq ID No. 4 and Seq ID No. 5.

Claim 31 (previously presented): The method according to claim 26, wherein the amplified fragments are detected by ethidium bromide staining or DNA probe hybridization.

Claim 32 (previously presented): The method as claimed in claim 26, wherein the step of differentiating comprises the steps of:

- a) designing a set of primers selected from the group consisting of Seq ID No. 1, Seq ID No. 2, Seq ID No. 3, Seq ID No. 4 and Seq ID No. 5 to amplify a part of said *hupB* gene from *Mycobacterium tuberculosis* and *Mycobacterium bovis*;
- b) obtaining DNA from culture or from clinical samples;
- c) amplifying a part of the target gene encoding for histone like proteins such as *hupB* of *Mycobacterium* species using said DNA as a template in a polymerase chain reaction with a pair of said oligonucleotide primers;
- d) analyzing and validating the size of the amplified fragment;
- e) determining the complete sequence of said amplified fragments; and
- f) inferring from the sequence whether it is *Mycobacterium tuberculosis* or *Mycobacterium bovis*.

Claim 33 (currently amended): The method according to claim 31, wherein the DNA probe consists of Seq ID No. 7 or Seq ID No. 8 or a complement thereof tagged with a detectable label.

Claim 34 (previously presented): The method as claimed in claim 26, wherein the step of differentiation consists in determining the smaller size of the amplified fragment obtained from *Mycobacterium bovis*.

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Claim 35 (previously presented): The method according to claim 28, wherein the PCR amplified fragment in *Mycobacterium bovis* is 618 bp.

Claim 36 (previously presented): The method according to claim 28, wherein the PCR amplified fragment in *Mycobacterium tuberculosis* is 645 bp.

Claim 37 (previously presented): The method according to claim 29, wherein the PCR amplified fragment in *Mycobacterium bovis* is 291 bp.

Claim 38 (previously presented): The method according to claim 30, wherein the PCR amplified fragment in *Mycobacterium tuberculosis* is 318 bp.

Claim 39 (previously presented): The method according to claim 29, wherein the PCR amplified fragment in *Mycobacterium bovis* is 89 bp.

Claim 40 (previously presented): The method according to claim 29, wherein the PCR amplified fragment in *Mycobacterium tuberculosis* is 116 bp.

Claim 41 (previously presented): The method according to claim 26, wherein the PCR amplified fragment in *Mycobacterium bovis* is 27 bp smaller than that of *Mycobacterium tuberculosis*.

Claim 42 (previously presented): The method as claimed in claim 26, wherein differentiating *Mycobacterium tuberculosis* and *Mycobacterium bovis* comprises the steps of:

- a) amplifying a part of the target *hupB* gene from *Mycobacterium tuberculosis* and *Mycobacterium bovis* in a polymerase chain reaction with primers Seq ID No. 1 and Seq ID No. 2;
- b) restricting the amplified fragment with *Hpa II* restriction enzyme to produce restricted fragments;
- c) separating the restricted fragments by electrophoresis on 12% polyacrylamide gel; and
- d) detecting the restricted fragments by staining with ethidium bromide.

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Claim 43 (previously presented): The method according to claim 42, wherein the restricted fragment in *Mycobacterium tuberculosis* is 280 bp and 150 bp.

Claim 44 (previously presented): The method according to claim 42, wherein the restricted fragment in *Mycobacterium bovis* is 253 bp and 150 bp.

Claim 45 (previously presented): A *hupB* gene comprised of Seq ID No. 8 as claimed in claim 25.

Claim 46 (previously presented): A *hupB* gene comprised of Seq ID. No. 7 as claimed in claim 25.